

### The Office Action

Claims 1-8, 10-13, 15-19, and 58 are pending. Claims 1-8, 10-13, 15-19, and 58 stand rejected for lack of written description and also for lack of enablement. In addition, claims 1, 15, and 18 stand rejected for anticipation by Knuth et al. (U.S. Patent No. 5,989,554; hereafter "Knuth").

### Submission of Formal Drawings

Applicant submits herewith formal drawings as requested by the Examiner.

### Rejections under 35 U.S.C. 112, first paragraph – Written Description

Claims 1-8, 10-13, and 15-19 are rejected for lack of adequate written description.

The Examiner states:

[T]he specification does not convey to the artisan that the applicant had possession, at the time of invention, of the claimed unstable polypeptide sequence (with the exception of the unstable polypeptide segment of the mobile loop of HSP10) comprised by a method for stimulating an immune response specific toward a naturally occurring protein...

The Examiner further asserts that the Applicant only exemplifies one unstable sequence, the mobile loop of Hsp10. This assertion is incorrect.

Applicant discloses many unstable sequences in the application, both inherently and explicitly. For example, Fig. 4 describes the location of flexible loops in staphylococcal nuclease, lysozyme, and cytochrome c, the sequences of which were known in the art. In addition, as described in the attached declaration by Dr. Samuel Landry, Applicant notes that Hsp10 is a family of compounds that have similar structural

features. Fig. 1B of Mande et al. (Science 1996, 271:203-207; hereafter "Mande"), of record, shows the aligned sequences for thirty-seven members of this family, including *Mycobacterium leprae*. The instant specification teaches in Fig. 1A that the mobile loop of *M. leprae* is at residues 19-35. Given this information, one skilled in the art could readily determine the appropriate sequence for the mobile loops of the various Hsp10 proteins. Thus, Applicant has described many species that fall within the claimed genus.

In addition, the invention is based on the discovery by the Applicant that the insertion of an unstable segment into a protein would increase the antigenicity of the C-terminal adjacent (or overlapping) segment. As described in the attached declaration, it is the flexibility of the inserted segment and not its primary sequence that is most important. Thus, in contrast to the Examiner's assertion, the recitation of factors that determine appropriate unstable sequences would convey to one skilled in the art that the applicant had possession of the invention, since the basis of the invention is the flexibility of the segments and not any particular sequence. The written description rejection should be withdrawn.

#### Rejections under 35 U.S.C. 112, first paragraph – Enablement

Claims 1-8, 10-13, and 15-19 are rejected for lack of enablement. Applicant respectfully disagrees.

The Examiner states:

It is known in the art that even a single amino acid change or difference in a protein's amino acid sequence can have dramatic effects on the protein's function, as evidenced by the teachings of Abaza et al... Further Hubbard

(IDS) teach in Protein Science (1994) that specific conformations are required for cleavage of limited proteolytic sites to enable the protease to bind and cut... Since antigenic epitopes are generated through proteolysis in the cell, a proper conformation must be achieved in the protein for proteolysis in the cell, and therefore inserting any instable segment may not lead to a proper conformation for proteolysis by endogenous proteases.

These arguments appear to assert that a particular sequence is necessary to achieve the proper conformation for proteolysis. Hubbard et al., however, state:

[L]arge local motions proximate to the scissile bond are required for proteolysis, and it is this ability to unfold locally without perturbing the overall protein conformation that is the prime determinant for limited proteolysis.” (abstract)

As is further described in the attached declaration, a particular peptide to be cleaved need not have a particular sequence, but rather needs to have the flexibility to conform to a particular conformation. The precise sequence is unimportant as long as the sequence is flexible. One skilled in the art would thus reasonably expect that any flexible polypeptide appropriately inserted would create a site that would exhibit preferential cleavage by the myriad proteases found endogenously in a lysosome. Applicant has used this property of unstable sequences to develop a method of increasing the antigenicity of a protein essentially by inserting proteolytic sites in that protein. The instant claims recite criteria, e.g., hydrophilicity and B-factors, to use in determining an appropriate unstable sequence for use in the claimed methods.

Furthermore, the attached declaration provides data on the insertion of sequences other than the mobile loop of the Hsp10 protein. These data indicate that insertion of a flexible sequence introduces proteolytic sensitivity in a protein. Since, as stated above, it is the conformational flexibility of the unstable polypeptide and not its precise sequence

that is important in the present invention, Applicant's disclosure on the criteria for determining an unstable sequence would enable one skilled in the art to practice the invention as claimed. The rejection of claims 1-8, 10-13, and 15-19 for lack of enablement should be withdrawn in view of the foregoing remarks.

Claim 58 stands rejected for lack of enablement. Applicant traverses this rejection.

The Office states:

There is insufficient guidance and direction regarding the sequence and structure of the human HSP-10 mobile loop inserted into the HIV gp120 protein.

Applicant notes in the attached declaration that the sequence of the human Hsp10 mobile loop was known in the art at the time the specification was filed. As stated above, Figure 1B in Mande shows an alignment of Hsp10 sequences from various organisms, including *M. leprae* and human. Given this information and the location of the mobile loop in *M. leprae* (residues 19-35, Fig. 1A of the specification), one skilled in the art could readily determine the appropriate sequence for the human Hsp10 mobile loop. The rejection of claim 58 may be withdrawn.

Rejections under 35 U.S.C. § 102(e)

Claims 1, 15, and 18 stand rejected for anticipation by Knuth. Applicant respectfully disagrees.

Claim 1 (from which claims 15 and 18 depend) recites:

1. A method for stimulating an immune response specific toward a naturally-occurring protein in an animal having an immune system including T cells, said method comprising administering to said animal an altered protein or polypeptide fragment thereof derived from said naturally-occurring protein, wherein an unstable polypeptide segment has been inserted by artifice into said altered protein, wherein said unstable polypeptide segment has an average hydrophobicity value that is lower than the average hydrophobicity value of said altered protein; has a sequence conservation that is lower than a sequence conservation of said altered protein; has an amide protection factor that is lower than  $10^4$  wherein said altered protein is in a native conformational state; has an average amide protection factor that is lower than the average amide protection factor for said altered protein in a denatured conformational state; has an NMR order parameter ( $S^2$ ) of less than 0.8; or has an average B-factor value that is higher than the average B-factor value of said altered protein, and wherein immunogenicity of said naturally-occurring protein is increased. (emphasis added)

Thus, claim 1 requires (1) a naturally occurring protein, or fragment thereof, and (2) an unstable polypeptide sequence. This unstable sequence is described by several characteristics, including an average hydrophobicity value lower, i.e., more hydrophilic, than that of the protein.

Knuth discloses a composition having essentially the opposite properties to those of the instant claims. For example, Knuth states:

The basic invention is directed to the construction of a carrier protein conjugate or a carrier protein complex. The carrier protein conjugate or complex comprises a first amino acid sequence, wherein the first amino acid sequence is substantially non-naturally occurring and insoluble, and a ligand having biological activity or immunological binding activity. (emphasis added) (col. 14, ll. 12-18)

In contrast to the more hydrophilic sequence inserted in Applicant's invention, the composition of Knuth employs an insoluble, i.e., hydrophobic, non-naturally occurring

polypeptide to which a ligand is bound. Thus, Knuth fails to teach or suggest the limitations of claims 1, 15, or 18, and the rejection for anticipation should be withdrawn.

### CONCLUSIONS

Applicant submits that the claims are now in condition for allowance, and such action is respectfully requested. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date:

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